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Survival of *Bacillus licheniformis* in Seawater Model Ecosystems

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The fate of *Bacillus licheniformis* DSM 13 was monitored after introduction into laboratory microcosms and mesocosms established in the Knebel Vig estuary, Denmark. The model organism was detected by a combination of immunofluorescence microscopy and nonselective plating followed by colony blotting. This allowed simultaneous quantification of intact cells and culturable cells. *B. licheniformis* DSM 13 adapted poorly to the conditions in filtered (0.2-μm-pore-size filter) seawater. Results from additional microcosm studies using natural seawater demonstrated that protozoan grazing also was important in regulating the population of the introduced model organism. In experiments using mesocosms, *B. licheniformis* DSM 13 also showed a rapid die-off. The introduction of the organism led to increased nutrient levels and to increased growth of both autotrophic and heterotrophic components of the plankton community compared with those of control enclosures. Thereby, a more intensive predation impact on the bacterioplankton community was induced. The combination of microcosm and mesocosm experiments provides a scenario in which the influence of single biotic and abiotic factors on survival of introduced organisms can be tested and in which the effect of the introduction on ecosystem structure and function can be evaluated. This test concept might prove useful in risk assessment of genetically modified microorganisms.

The human exploitation of genetically engineered microorganisms (GEMs) involves the contained use of GEMs in biotechnological production as well as the deliberate release of GEMs into the environment. Thus, planned or accidental release into the environment is or may be a consequence of using these organisms. The potential risk associated with the release of a GEM depends on the ability of the organism to become established in the environment (44). Consequently, it is important to identify factors regulating bacterial growth and survival in natural environments and to evaluate the effects of release on ecosystem structure (45).

An increased understanding of bacterial survival requires methods for detecting specific phenotypes or genotypes in the environment and model ecosystems in which the fate of the relevant organism can be studied.

Immunochemical methods based on antibodies against surface-exposed epitopes have primarily been used in studies of naturally occurring bacterial populations (4, 24). In recent years, detection methods for genotypes based on DNA probes have been developed to demonstrate naturally occurring or genetically modified bacteria in the aquatic environment (9, 27, 42). However, antibodies can be directed against the product of a recombinant gene, and immunochemical methods can consequently be used parallel with DNA probes to monitor the fate of released GEMs (31). Importantly, the fate of bacteria that are not culturable but possibly are ecologically relevant (39) may be approached by direct methods based on DNA-DNA or antibody-antigen recognition.

Microcosms established in the laboratory have proven useful for evaluating the effects of single factors such as salinity, light, and predation on the survival of a model organism in experiments of short duration. Several studies of the survival of pathogens and of *Escherichia coli* have been performed with freshwater or seawater microcosms (15, 18, 23, 30). Recently, microcosms have been used to study the survival of recombinant microorganisms (1, 6, 40).

So far, mesocosms have not been applied in studies of the ecological effects of introduced bacteria, although mesocosms have proven to be a very useful tool in studies of natural populations of microorganisms (10, 37, 38).

In the present study, microcosm and mesocosm experiments were combined to evaluate the fate of an introduced microorganism. *Bacillus licheniformis* was used as a model organism in these experiments, as genetically modified strains of *B. licheniformis* are of potential use in contained biotechnological enzyme production (16, 20).

The objective of this study was to simulate a step-by-step risk assessment by (i) comparing the survival of *B. licheniformis* in the two model ecosystems and (ii) assessing the effects of the release of the organism on the natural population structure.

MATERIALS AND METHODS

Bacterial strain. *B. licheniformis* Deutsche Sammlung von Mikroorganismen no. 13 (DSM 13) was used as a model organism in this study. Prior to inoculation into model ecosystems, DSM 13 was grown in Luria-Bertani (LB) medium at 20°C with vigorous shaking to the late logarithmic phase. These cultures consisted of vegetative cells.

Culturability of DSM 13 was determined by culturing relevant dilutions on nutrient agar (Difco) for 3 days at 20°C and then by identification of colonies of DSM 13 by colony blotting (see below).

Immunochemical detection methods. *B. licheniformis* DSM 13 was detected with a polyclonal rabbit antibody raised against heat-denatured whole cells harvested in the early stationary phase. The immunization dose was 2 mg per injection, and immunizations were performed by DAKO A/S (Copenhagen, Denmark) according to the protocol described by Harboe and Ingild (21). The antibody reacted strongly with both vegetative cells and spores in cultures of *B.*

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licheniformis DSM 13. It showed weak cross-reactions to bacteria occurring in the experimental area.

Samples for immunofluorescence microscopy were fixed with glutaraldehyde and filtered through 0.2-μm (pore size) black polycarbonate membranes (Nuclepore). The filters were blocked for 10 min in blocking buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 2% Tween 20, 0.5% bovine serum albumin [BSA]) and washed three times with 5 ml of washing buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.05% Tween 20, 0.05% BSA). Filters were then incubated for 1 h with rabbit antiserum diluted 1:400 in dilution buffer (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 0.05% Tween 20, 0.5% BSA), washed as described above, and incubated for 1 h with fluorescein isothiocyanate-conjugated swine anti-rabbit immunoglobulins (DAKO) diluted 1:40. Cells of *B. licheniformis* DSM 13 labelled with fluorescent antibody (FA) were counted (FA counts) in a fluorescence microscope (Olympus).

Colony-blotting procedures were modified from the methods of Olsen and Rice (34). Nitrocellulose filters (BA 85; Schleicher & Schuell) were placed on plates containing 50 to 200 colonies and allowed to wet completely. Filters were then removed and washed before being blocked and incubated with rabbit antiserum (1:400) overnight. Incubation with alkaline phosphatase-conjugated swine anti-rabbit immunoglobulins (DAKO) diluted 1:1,000 was done for 1 h. Enzyme staining was performed for 10 min with nitroblue tetrazolium (Sigma) and 5-bromo-4-chloro-indolylic phosphate (Boehringer) as substrates. Buffers were used as described above, except that a pH of 10.2 was used as described by Nybroe et al. (33).

Inoculation of model ecosystems. The mesocosms and some of the microcosms were inoculated with crude culture containing ca. 10^9 cells ml^{-1}. Other microcosms were inoculated with cells that had been harvested by centrifugation at 6,000 × *g* for 5 min, washed twice in 0.85% NaCl, and resuspended at 10^9 cells ml^{-1} in 0.85% NaCl. The cell number of the inoculum was determined as acridine orange direct counts (AODC; see below) immediately before inoculation. The concentration of *B. licheniformis* DSM 13 in the model ecosystems was 10^6 cells ml^{-1} at the start of experiments. Hence, inoculum volume was 1‰ of the total model ecosystem volume.

Microcosm experiments. Microcosms were established in 500-ml conical flasks containing natural seawater or seawater that had been sequentially filtered through a GF/C filter and a 0.2-μm-pore-size filter. The water used for the experiments was collected, either at a near-shore station in Øresund (salinity, 10‰; NO_2-N plus NO_3-N, 45 μg $liter^{-1}$; NH_4-N, 35 μg $liter^{-1}$; PO_4-P, 20 μg $liter^{-1}$ [31a]) or close to the field station in Knebel Vig (salinity, 20‰; NO_2-N plus NO_3-N, 45 μg $liter^{-1}$; NH_4-N, 20 μg $liter^{-1}$; PO_4-P, 8 μg $liter^{-1}$ [41a]). Microcosms were incubated at 20°C with gentle shaking in the dark. Inoculation and all subsequent manipulations of the microcosms were performed aseptically. Microcosm experiments using water from Øresund were performed in duplicate.

Mesocosm experiment. Enclosure experiments were carried out in Knebel Vig in September 1990. Knebel Vig is a small embayment of the east coast of Kalø Vig, which is a part of the Bay of Århus (Fig. 1). Knebel Vig has a maximum depth of 16 m and a surface area of 37 km^2. Seawater was filled into six transparent, cylindrical plastic enclosures (1.5 m in diameter and 4 m deep) by pulling the top part of submerged enclosures toward the water surface. The enclosures were closed just above the sediment surface and fixed to a pontoon bridge (Fig. 1). The water in the enclosures was kept in circulation by wind-driven mills. Two enclosures were inoculated with *B. licheniformis* DSM 13, and two enclosures served as untreated controls.

Water samples were collected from the enclosures by integrating 5-liter samples from three depths in each enclosure prior to analyses. The first samples were taken 1.5 h after inoculation to ensure proper mixing.

Determination of bacterial colonization of the plastic surface was monitored by plastic strips placed at a 0.5-m depth at the start of the experiment. Approximately 1 cm^2 was cut off the strips at days 2 and 5 and placed in distilled water containing 1% formaldehyde. Later, each sample was sonicated for 8 min on ice with a Branson Sonifier (duty cycle 10, output level 1) to release bacteria. Microscopic inspection of the plastic after sonication showed that only a few bacteria were still attached to the surface after the treatment. The total number of bacteria in the released material was determined as AODC (see below), and the number of DSM 13 was determined as FA counts.

At each sampling date, water samples were transported to the laboratory at 4°C for determination of culturability. The temperature and oxygen content at three depths (0.5, 1.5, and 2.5 m) were determined with an oxygen/temperature probe (model YSI 58; Yellow Spring Instruments) before the withdrawal of water samples.

Other experimental procedures. (i) Nutrients. Inorganic phosphate (PO_4-P) and ammonia (NH_4-N) were analyzed according to the methods of Murphy and Riley (32) and Crosby (12), respectively.

(ii) Bacteria. The total bacterial population was enumerated by epifluorescence microscopy by using the standard AODC procedure (Hobbie et al. [22]), and cells of *B. licheniformis* DSM 13 were enumerated by immunofluorescence microscopy (FA counts, see above).

Bacterial net production was determined by means of [^{3}H]thymidine incorporation into ice-cold trichloroacetic acid precipitate according to the procedure of Fuhrman and Azam (17) as modified by Riemann (35). A conversion factor of 1.1 × 10^{18} cells mol^{-1} of thymidine incorporated was applied to convert thymidine incorporation rates into the number of cells produced (36, 41).

(iii) Phytoplankton. For determination of chlorophyll *a*, duplicate samples were filtered onto 25-mm GF/C filters and extracted in 96% ethanol for 20 h without homogenization as described by Jespersen and Christoffersen (26). The pigment extract was purified by high-pressure liquid chromatography (HPLC) by using HPLC equipment (Shimadzu) with a lichrosorb RD-18 column. Chlorophyll *a* was measured in a fluorometer (Shimadzu) calibrated with chlorophyll *a* purified by paper chromatography (25). To calculate chlorophyll *a* concentrations, a specific absorption coefficient of 83.4 liters g^{-1} cm^{-1} was applied (46).

Samples for species identification were fixed in acid Lugol's solution.

(iv) Zooplankton. Heterotrophic nanoflagellates were enumerated from proflavine-stained samples by epifluorescence microscopy (19). Ciliates and mesozooplankton were identified and counted in Lugol-fixed samples with an inverted microscope and a stereomicroscope, respectively.

RESULTS

Survival in microcosms containing filtered (0.2-μm-pore-size filter) water. Results from survival experiments performed in microcosms containing water from Øresund and

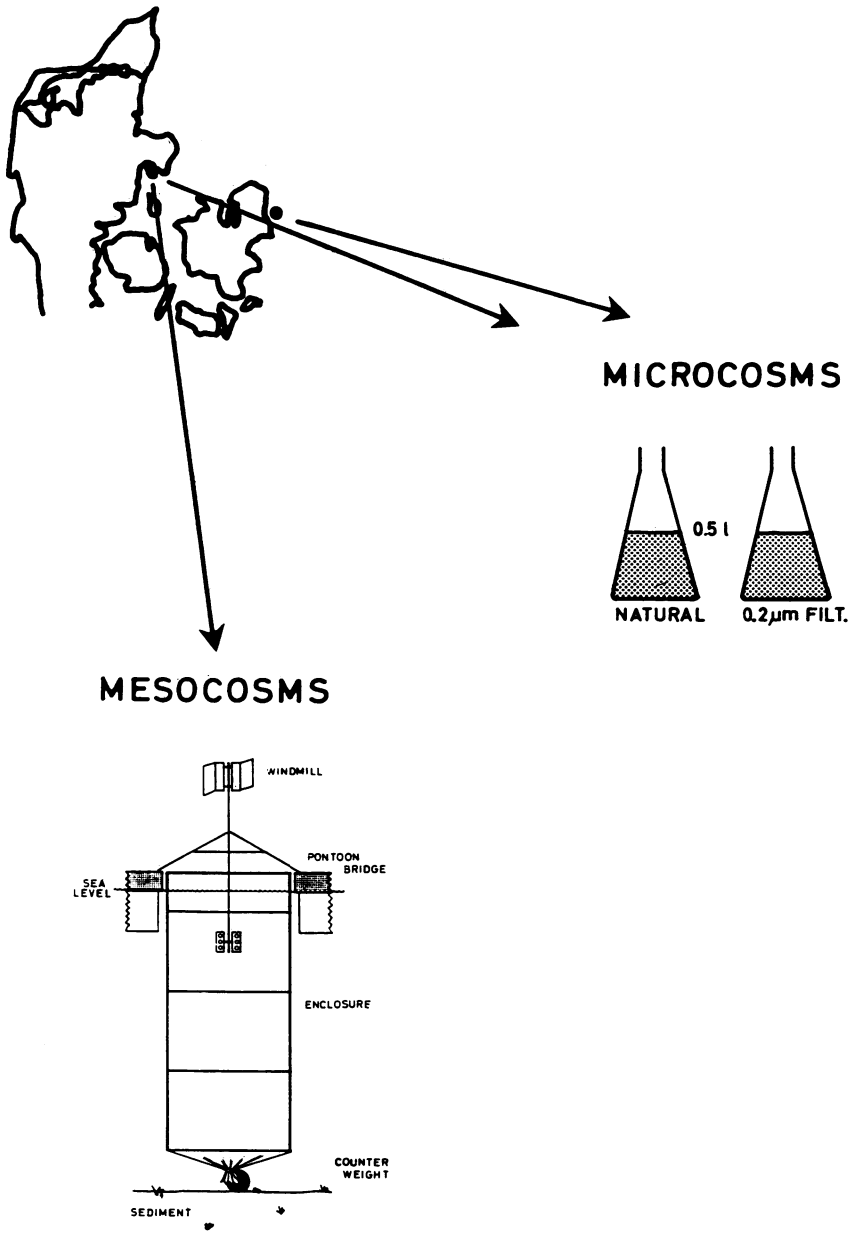

FIG. 1. Sampling locations and an outline of the model ecosystems employed.

inoculated with washed cells of *B. licheniformis* DSM 13 showed that after a lag phase of 2 days, the total number of bacterial cells, determined as AODC, increased gradually during the rest of the experimental period (Fig. 2A). In contrast, FA counts of DSM 13 declined during the same period. In all experiments presented here, no signs of sporulation of *B. licheniformis* DSM 13 could be detected. *B. licheniformis* DSM 13 viable counts corresponded to FA counts immediately after inoculation but then decreased about 3 log units during the 8-day experimental period. The T_{90} value, expressing the time after which 90% of the added population of bacteria was no longer culturable, was ca. 4 days (Fig. 2A).

In a parallel experiment, microcosms were inoculated with crude culture. The general pattern was the same as that in the previous experiment (Fig. 2B). However, the increase in total bacterial population was higher, and FA counts of *B. licheniformis* DSM 13 were stable rather than declining. The decrease in culturability of DSM 13 was smaller, the T_{90} value being ca. 6 days.

Microcosms containing water from Knebel Vig and inoculated with crude culture showed very poor survival of DSM 13. FA counts decreased by 80% in 5 days, and viable cells disappeared completely during the same period (Fig. 3A). Furthermore, lysed cells of *B. licheniformis* DSM 13 could be observed in this system.

Survival in microcosms containing natural water. In microcosms containing natural water from Øresund and inoculated with either washed cells or crude culture, the total bacterial population increased during the first day but then stabilized

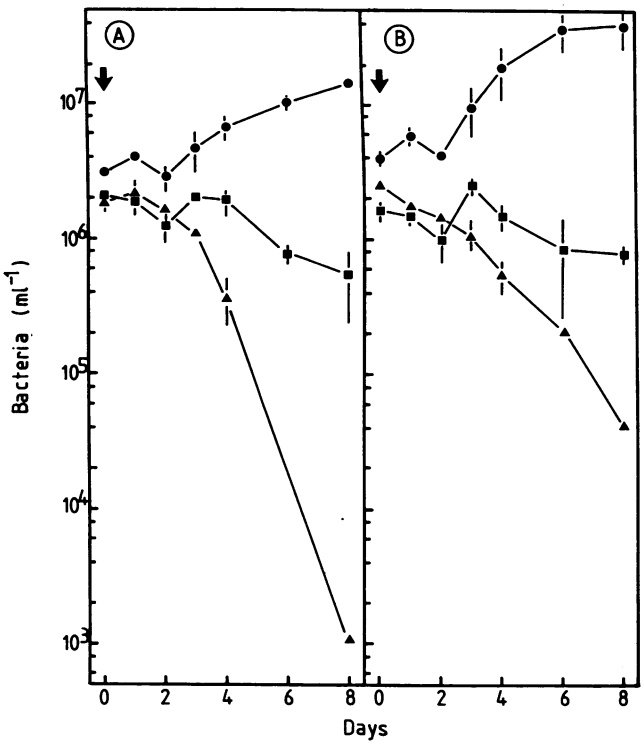

FIG. 2. Survival of *B. licheniformis* DSM 13 in microcosms containing filtered (0.2-μm-pore-size filter) water from Øresund. Microcosms were inoculated with washed cells (A) and with crude culture (B). ●, total bacteria measured by AODC; ■, DSM 13 measured by FA counts; ▲, DSM 13 measured by being plated on nutrient agar and then by colony blotting. The arrows indicate the time of inoculation; bars indicate the ranges of results from duplicate microcosms.

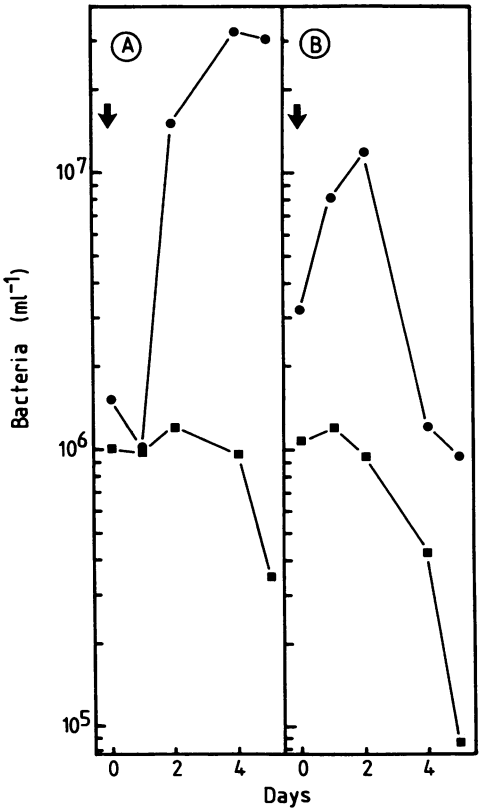

FIG. 3. Survival of *B. licheniformis* DSM 13 in microcosms containing water from Knebel Vig. (A) Filtered (0.2-μm-pore-size filter) water; (B) natural water. Microcosms were inoculated with crude culture. ●, total bacteria measured by AODC; ■, DSM 13 measured by FA counts. The arrows indicate the time of inoculation.

at a slightly lower level. The FA counts of *B. licheniformis* DSM 13 decreased by ca. 2 log units (Fig. 4), and viable counts dropped precipitously to levels below the detection limit (10 CFU ml^{-1}) during the experimental period, the T_{90} being less than 2 days (Fig. 4).

The population size of heterotrophic nanoflagellates in the microcosms increased from ca. 4×10^2 to ca. 1×10^5 during the first 2 days and then gradually declined. The increase was largest in microcosms inoculated with crude culture (Fig. 4).

In microcosms with natural water from Knebel Vig, the total bacterial population as well as DSM 13 increased during the first 1 to 2 days after inoculation but then decreased, as seen with the Øresund experiment (Fig. 3B). Viable counts of *B. licheniformis* DSM 13 disappeared in less than 5 days.

Survival in mesocosms. The total number of bacteria in the water phase increased during the first 3 days by a factor of 2.7 and 1.4 for inoculated and control enclosures, respectively (Fig. 5A). A subsequent decrease was recorded for all enclosures. Bacterial cell production was initially ca. 20 times higher after introduction of *B. licheniformis* DSM 13 than that in control enclosures but decreased within 4 days to control levels (Fig. 5B). A further decrease was found for all enclosures during the last part of the experiment.

B. licheniformis DSM 13 in inoculated enclosures could only be quantified by FA counts at day 0 and day 1 (Fig. 5A). DSM 13 was also observed at day 2 but in numbers too low to allow reliable quantification ($\ll 5 \times 10^4$ ml^{-1}). Lysed cells were observed from day 1 as in the case of the microcosms containing water from Knebel Vig. Culturable *B. licheniformis* DSM 13 could be demonstrated at day 0 only in a concentration of 2×10^4 ml^{-1}, which indicated that culturability was low soon after inoculation.

The biofilm (simulated by plastic strips) consisted of 8×10^5 bacteria per cm^2 at day 2 and 4×10^5 bacteria per cm^2 at day 5; however, DSM 13 could not be detected in these samples.

The temperature decreased from 14.3 to 11.2°C during the experiment. Meanwhile, the oxygen content increased from 7.2 to 9.6 mg of O_2 liter^{-1}. There was no difference in temperature and oxygen content between the enclosures or between the enclosures and the surrounding water.

Inorganic phosphate averaged 7 μg of PO_4-P liter^{-1} during the experimental period in the enclosures inoculated with DSM 13 but decreased from 7 to 2 μg of PO_4-P liter^{-1} in the enclosures without DSM 13 (Fig. 6A). Ammonium was initially high (>300 μg of NH_4-H liter^{-1}) in the enclosures with DSM 13 but decreased rapidly to less than 10 μg of NH_4-H liter^{-1} as was found in the control enclosures (Fig. 6B).

The level of chlorophyll *a* (Fig. 6C) was higher in the enclosures containing *B. licheniformis* DSM 13 than in control enclosures. The phytoplankton community was dominated by different *Chaetoceros* species in all enclosures.

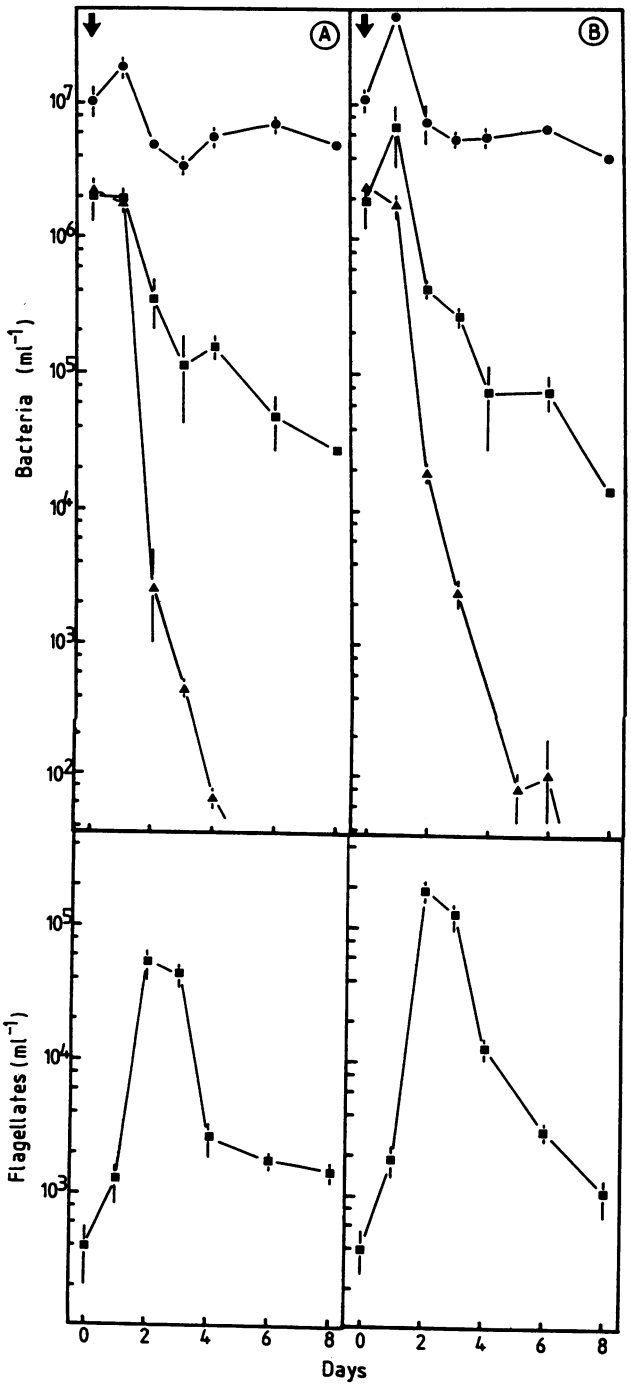

FIG. 4. Survival of *B. licheniformis* DSM 13 in microcosms containing natural water from Øresund. Microcosms were inoculated with washed cells (A) and with crude culture (B). Upper panel: ●, total bacteria measured by AODC; ■, DSM 13 measured by FA counts; ▲, DSM 13 measured by being plated on nutrient agar and then by colony blotting. Lower panel: ■, number of heterotrophic flagellates. The arrows indicate the time of inoculation; bars indicate the ranges of results from duplicate microcosms.

Heterotrophic nanoflagellates increased in numbers in the enclosures with DSM 13 and peaked at 3.2×10^3 flagellates ml^{-1} 5 days after the addition (Fig. 6D). Thereafter, the numbers decreased to 1×10^3 to 2×10^3 ml^{-1}. The grazing impact of the heterotrophic nanoflagellates was calculated in two ways: on the basis of (i) a flagellate clearance rate of 5 nl individual^{-1} h^{-1} (38) and (ii) specific clearance rates (10^5 body volumes cleared per unit of time). Results from one enclosure (Table 1) showed that the two calculations gave comparable results. The grazing pressure was highest at day 5 when 40 to 50% of the bacterial population was removed per day. The number of flagellates in the enclosures without DSM 13 ranged between 0.5×10^3 ml^{-1} and 1.5×10^3 ml^{-1} throughout the period, and flagellate grazing removed ca. 13% of the bacteria per day.

The number of ciliates decreased slightly during the experiment to 3.0×10^3 liter^{-1} in enclosures with DSM 13 and 1.5×10^3 liter^{-1} in the enclosures without DSM 13 at the end of the experiment (Fig. 6E). The ciliates were dominated by *Strombidium* and *Mesodinium* spp. in all enclosures.

The zooplankton community consisted of different copepod species and benthos larvae. The total number fluctuated between 5 and 12 individuals liter^{-1} in all enclosures, with a tendency to increased numbers in the enclosures inoculated with DSM 13 (Fig. 6F) toward the end of the experiment.

DISCUSSION

In the present study, a combination of microcosm and mesocosm experiments was used to evaluate the survival of an introduced microorganism. In each system, the model organism was monitored by immunofluorescence microscopy and by nonselective plating combined with colony blotting, thus allowing the persistence of intact cells to be monitored in parallel with the culturability of the organism. Methods based on plating on general medium followed by colony blotting have been reported to underestimate the population size of model organisms because of competition from the indigenous bacteria in freshwater systems (1, 42). This type of problem was not encountered in the present experiments, as culturable counts of the indigenous population were low, typically less than 5×10^2 ml^{-1}.

Initially, microcosm experiments were performed in filtered (0.2-μm-pore-size filter) seawater to exclude the effect of protozoan predation. In these microcosms, a proliferation of indigenous bacteria was detected. This proliferation could originate from cells of <0.2 μm in size (29) or from larger bacteria which had passed through the 0.2-μm-pore-size filters (5, 43). In contrast, *B. licheniformis* DSM 13 was not able to become established in the water samples employed. Nutrients originating from the inoculation with crude culture, as opposed to washed cells, improved the survival of DSM 13 only marginally (Fig. 2). Survival was poorer in water from Knebel Vig than in water from Øresund (Fig. 2 and 3). The water from Knebel Vig had a lower nutrient content and a higher salinity than water from Øresund. Hence, a combination of these factors might affect survival of DSM 13.

In general, *B. licheniformis* DSM 13 had poorer survival in sterile filtered seawater than that reported for *E. coli* (15, 30). Survival was also low compared with survival of *E. coli* and *Pseudomonas* spp. in sterile filtered lake water (1, 11, 31) but comparable with that of *Bacillus subtilis* CU155 known to be susceptible to starvation (18).

Comparison of survival curves for microcosms with filtered and natural seawater demonstrated that protozoan

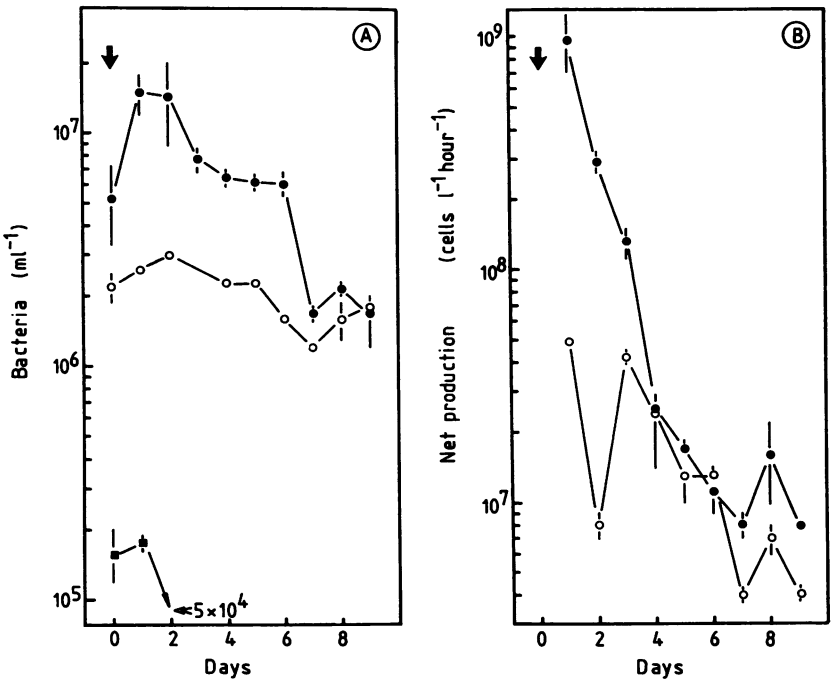

FIG. 5. (A) Survival of *B. licheniformis* DSM 13 in Knebel Vig mesocosms. Crude culture was used as an inoculum. ●, total bacteria in inoculated enclosures measured by AODC; ○, total bacteria in control enclosures measured by AODC; ■, DSM 13 measured by FA counts. (B) Bacterioplankton production in mesocosms inoculated with DSM 13 (●) and in control mesocosms (○). The arrow indicates the time of inoculation; bars give the ranges of results from duplicate mesocosms.

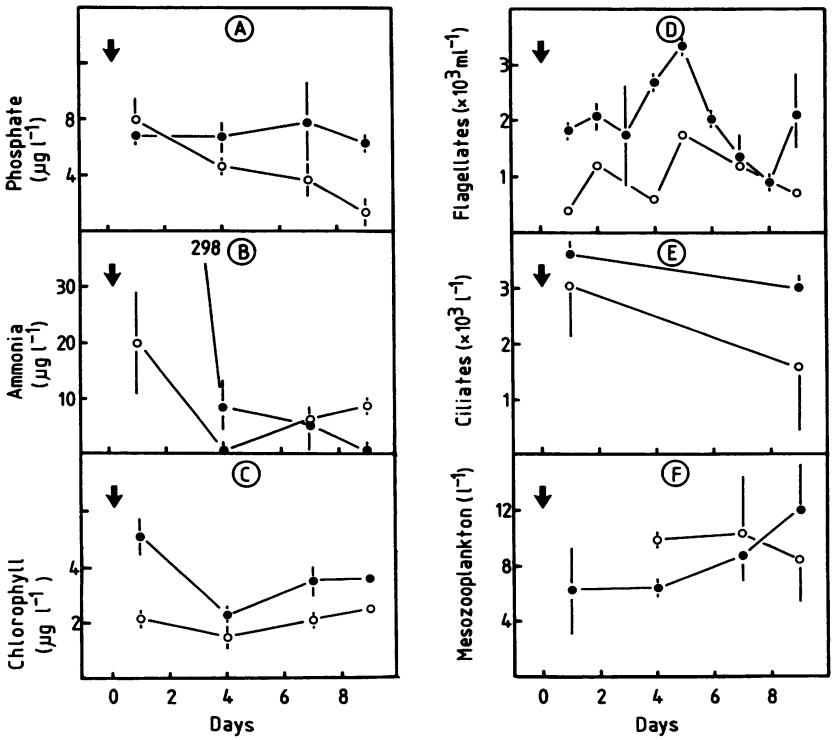

FIG. 6. Phosphate, ammonia, chlorophyll *a*, flagellates, ciliates, and mesozooplankton in mesocosms inoculated with *B. licheniformis* DSM 13 (●) and in control mesocosms (○). The arrows indicate the time of inoculation, and bars give the ranges of results from duplicate mesocosms.

TABLE 1. Clearance of bacteria by heterotrophic nanoflagellates in a water enclosure inoculated with *B. licheniformis* DSM 13 at day 0

Day	No. of flagellates ml^{-1}	Flagellate vol (μm^3)	Clearance[a] (% day^{-1})	Clearance[b] (% day^{-1})
1	1,806	102	22	44
5	5,359	63	40	50
9	1,613	75	20	29

[a] Clearance of bacteria based on a fixed individual clearance rate of 5 nl $individual^{-1}$ h^{-1}.
[b] Clearance of bacteria based on body volumes.

predation may have been an important regulator of the introduced organism (Fig. 2, 3, and 4). This is consistent with the observed increase in heterotrophic nanoflagellates (Fig. 4).

In mesocosms, estimates of flagellate grazing potential suggested that grazing by flagellates could be an important cause of bacterial mortality. Clearance rates of natural populations of flagellates range from 2 to 10 nl $individual^{-1}$ day^{-1} (2, 3, 10, 28, 38). This is apparently a much narrower range than that reported for cultured flagellates (13, 14) and indicates that our calculated community grazing rates are realistic. Recently, Gurijala and Alexander (18) found that protozoan predation was an important factor explaining the decline of several introduced microorganisms in freshwater microcosms.

In spite of the lower grazing pressure in the mesocosms compared with that in microcosms, survival of *B. licheniformis* DSM 13 in mesocosms was poorer than predicted from the microcosm experiments. As the water in the mesocosms was constantly mixed, sedimentation is not considered to be responsible for the decrease in DSM 13 cell numbers. Furthermore, colonization of the plastic surfaces of the enclosures could not be demonstrated. A major difference between survival experiments performed in the laboratory microcosms and in the field mesocosm was light conditions. Light is an important factor affecting survival of (e.g.) *E. coli* (7, 8), and we cannot rule out the possibility that light also influenced survival of DSM 13.

The addition of *B. licheniformis* DSM 13 led to increased nutrient levels (Fig. 6A through C) caused by the addition of bacterial cell cultures and subsequent predation or cell lysis. A consequence of the enhanced nutrient availability was increased growth of both autotrophic and heterotrophic components of the plankton community.

Bacterial biomass and net productivity increased immediately by a factor of 39 and 20, respectively, in enclosures inoculated with DSM 13 (Fig. 5). This was followed some days later by a marked increase in the number of heterotrophic nanoflagellates (Fig. 6D), which induced a more intensive predation impact on the bacterioplankton. Slowly growing organisms like the mesozooplankton showed only a weak response in the last part of the experimental period (Fig. 6F). The short duration of the experiments probably did not allow a full appreciation of the grazing potential of the mesozooplankton. Similar scenarios of interactions within natural plankton populations upon nutrient enrichments have been reported by Bjørnsen et al. (10) and Riemann et al. (37, 38).

In the present study, microcosm experiments indicated that *B. licheniformis* DSM 13 die-off was caused partly by the inability of the organism to adapt physiologically to seawater. Moreover, results from microcosm studies showed that protozoan predation could be an important factor regulating its population size. These findings were in agreement with the poor survival in mesocosms. However, the rapid and dramatic increase in heterotrophic nanoflagellates in the microcosms was less pronounced in the mesocosms. This indicates that changes in ecosystem structure caused by the introduction of a foreign microorganism have to be analyzed in more realistic model ecosystems.

In conclusion, microcosms seem useful for studying the effect of single factors, such as nutrients, salinity, and predation, on the survival of an introduced organism. However, integrated effects of the continuum of biotic and abiotic factors affecting survival in a natural plankton community cannot be simulated in simple microcosm experiments. For this purpose, a dynamic system under more realistic conditions is required. Water enclosure mesocosm experiments lasting up to several weeks seem to fulfill these requirements. Furthermore, mesocosm experiments allow studies of the effect of introducing a foreign microorganism on the structure of the aquatic ecosystem and on ecosystem function. Thus, the combination of aquatic microcosms and mesocosms provides a useful test scenario for studies of the colonization potential of introduced microorganisms. This test concept might prove useful in risk analysis of GEMs.

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